THYROID HORMONE TREATMENT AND OXYGEN CONSUMPTION IN EMBRYOS OF THE SPINY DOGFISH [1]

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The inability of adult cold-blooded vertebrates to respond to thyroxine treatment with an increased oxygen consumption rate is now a well documented finding (Hoar, 1957; Gorbman, 1959). The two often cited exceptions to this general experience are the thyroxine-induced increases in oxygen consumption in adult goldfish observed by Müller (1953), and in parrot fish of certain sizes, as described by Smith and Matthews (1948). Both of these claims have been denied by opposite results in the same species (Etkin, Root and Mofshin, 1940; Chavin and Rossmore, 1956; Matty, 1957). Measurements of metabolic rate in fishes are subject to numerous variables which are not as easily controlled as they are in mammals (responses to handling, previous temperature history, illumination, endogenous activity cycles) (Fry, 1957), so that it is not surprising that conflicting claims may exist for some species. Of the factors which may contribute misleading information in measurements of oxygen consumption in fishes, among the most significant is muscular activity. Hoar (1958) has shown clearly that treatment of fishes with thyroid hormone induces behavioral changes, expressed primarily by an increased spontaneous motor activity. If this is so, then any valid test for basal metabolic stimulation by thyroxine must exclude the variable of locomotor muscular work. Although testing systems are available which make this possible (Fry, 1957), neither of the two exceptional claims mentioned above utilized them.

While working with near-term embryos of the spiny dogfish, *Squalus suckleyi*, removed from the uterus and kept in flowing sea water, we noticed a behavioral feature which makes this animal useful in respiratory studies. When kept in subdued light they remain still, even after treatment with thyroid hormone. Since under these circumstances, spontaneous muscular movements are rare, then respiratory measurements can be taken to reflect "basal" requirements (or at least "standard" metabolism as defined by Fry, 1957), not a thyroxine-induced increase in swimming. In these experiments oxygen consumption of such exteriorized dogfish pups was measured after treatment with thyroxine, or two of its analogues, or propyl thiouracil. To our knowledge the only other studies of the metabolic responsiveness to thyroid hormone in larval vertebrates have concerned anuran tadpoles. In this regard, too, the published literature is in disagreement (Etkin, 1955; Lewis and Frieden, 1959). Accordingly, it was hoped that the experiments

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with dogfish embryos would prove enlightening, both with regard to the metabolic action of thyroid hormone in poikilotherms and its action in differentiating forms of such animals.

Material and Methods

Oxygen consumption was measured in a continuous-flow apparatus of the type used by Job (1955) in measuring the "standard" metabolism of trout. Four respirometer flasks (2.5-liter Fernbach culture jars) were used at any one time, one of these being used as a "blank." The four flasks were immersed in a large wooden tank in which the water level was maintained constant by means of an overflow. The flow of water through the flasks was so adjusted that *Squalus* embryos in groups of three removed about 0.5 to 1.0 ml. of oxygen. Rate of oxygen consumption was calculated from the flow rate, the difference in oxygen content between incurrent and excurrent water, and the wet weight of the animals

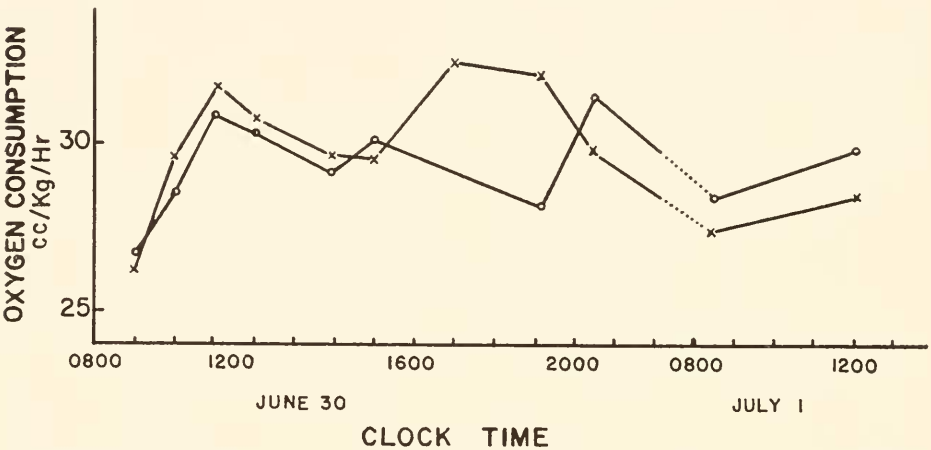

Figure 1. Serial determination of metabolic rate in untreated embryos of *Squalus sucklcyi* over a 27-hour period. Two respirometers used with three pups in each.

tested. Oxygen content of the water was determined by the unmodified Winkler technique.

Water temperature never varied more than 1° C. during a single series of tests, and usually it did not change at all measurably. During most measurements of oxygen consumption the water temperature ranged from 13° to 14° C. However, extremes of 12° C. and 16° C. (on one unusually hot day) were recorded. The water in the large water bath was continuously aerated through a stone "air breaker," and preliminary tests showed that the oxygen content of the water was uniform at all points in the bath.

Embryos were tested in groups of three, being placed in the respirometers one hour before the first measurement of oxygen consumption. The bath containing the respirometers was covered to shield the animals from most of the light and other extraneous factors. Frequent observation indicated that under these conditions spontaneous muscular movements were rare. Several prelimi-

TABLE I

Metabolic rates of non-treated dogfish pups showing normal day-to-day variability in the laboratory. All runs made between 1:00 and 3:00 PM

Date	No. of trials	Mean O_2 consumption (cc./kg./hr.)
July 3	4	28.8 ± 1.4 (std. dev.)
July 4	4	31.4 ± 1.9
July 6	3	29.5 ± .89
July 8	3	31.3 ± 1.4
July 10	3	33.7 ± 1.7

nary measurements were made on untreated pups, to ascertain the variability in oxygen consumption under laboratory conditions. Figure 1 illustrates the results of serial determinations on two groups of embryos, all taken from the same female, over a 1½-day period. The period of steadiest metabolic rate was in the afternoon; accordingly all measurements reported here were made in the afternoon only. In another preliminary test, metabolic rates were determined on pups from one female dogfish, over a period of one week immediately following transfer of the pups to the laboratory. The results (Table I) show that the average metabolic rate remained at about the same level over this period and that the variability in oxygen uptake (as indicated by the standard deviations) was quite small.

TABLE II

Experimental protocol for each injection group. Numbers in parentheses indicate the number of pups from that female

Experiment	Total no. of injections	Females from which pups were taken	Substances tested*	Dose per injection
1	10	A(15)	triac	10 μg.
			l-Tx	10 μg.
2	9	B(11), C(9)	triac	10 μg.
			l-Tx	10 μg.
			PTU	50 μg.
3	9	D(10), E(10)	triac	10 μg.
				100 μg.
			l-Tx	10 μg.
			PTU	50 μg.
4	8	F(11), G(4), H(4), I(4), J(5), K(7)	triac	10 μg.
				100 μg.
			l-Tx	10 μg.
			T3	100 μg.
			PTU	50 μg.
5	5	L(19)	l-Tx	100 μg.
			T3	100 μg.
			PTU	50 μg.
6	4	M(25), N(10)	triac	10 μg.
			l-Tx	10 μg.

* Abbreviations: triac, triiodothyroacetic acid; l-Tx, l-thyroxine; PTU, propylthiouracil; T3, triiodothyronine.

TABLE III

Effects of repeated injections of thyroid hormones on oxygen consumption (cc./kg./hr.) of embryos of Squalus suckleyi.* *Values in the table are means of 3 to 6 consecutive determinations, taken at 15-minute intervals. E = experimental. C = control*

Experiment	No. of injections		Triac 10 µg.	Triac 100 µg.	l-Tx 10 µg.	l-Tx 100 µg.	T3 100 µg.	PTU 50 µg.
1	1	E	32.40		33.52			
		C	30.79		32.52			
		E/C × 100	105		103			
	4	E	52.25		44.78			
		C	39.28		39.28			
		E/C × 100	133		114			
	9	E			46.82			
		C			44.60			
		E/C × 100			105			
2	2	E	32.25		32.15			28.78
		C	31.52		31.52			31.52
		E/C × 100	102		102			91
	4	E	41.90		40.90			36.00
		C	35.80		35.80			35.80
		E/C × 100	117		114			101
	9	E	33.80		41.40			31.27
		C	34.16		34.16			34.16
		E/C × 100	99		121			91
3	1	E	33.10	33.40	33.30			32.70
		C	33.20	33.20	33.20			33.20
		E/C × 100	99	101	100			98
	4	E	36.70	33.90	29.20			29.00
		C	30.40	30.40	30.40			30.40
		E/C × 100	121	108	96			98
	9	E	35.87	35.50	33.62			38.09
		C	32.96	32.96	32.96			32.96
		E/C × 100	109	108	102			115
4	2	E	34.13	32.56	30.82		30.12	30.30
		C	29.86	29.86	29.86		29.86	29.86
		E/C × 100	114	109	103		101	102
	5	E	37.02	33.45	33.97		34.63	32.51
		C	30.41	30.41	30.41		30.41	30.41
		E/C × 100	122	110	112		114	107
	8	E	32.33	33.66	31.86		32.28	29.44
		C	27.49	27.49	27.49		27.49	27.49
		E/C × 100	118	122	116		118	107

* This table includes absolute values for oxygen consumption at the beginning (after 1 or 2 injections), the middle (after 4 or 5 injections), and end after 8 or 9 injections in each experiment. The complete course of each experiment is shown in Figures 4A, 4B and 4C, but these do not show absolute values. To tabulate all the absolute values would require an impractically long table.

TABLE III—*Continued*

Experiment	No. of injections		Triac 10 μg.	Triac 100 μg.	l-Tx 10 μg.	l-Tx 100 μg.	T3 100 μg.	PTU 50 μg.
5	1	E				30.01	25.78	26.96
		C				26.86	22.82	22.82
		E/C × 100				112	113	118
	5	E				39.92	42.96	37.07
		C				35.78	35.78	35.78
		E/C × 100				112	120	104
6	1	E	28.49		30.37			
		C	26.18		26.18			
		E/C × 100	109		110			
	4	E	35.34		32.82			
		C	29.78		29.78			
		E/C × 100	119		110			

The animals used were "pups" removed from the uteri of *Squalus suckleyi* females caught during July and August, 1958, at Friday Harbor, Washington, within 200 yards of the laboratory. The ovoviviparous young of this species remain in the uterus for two years. It could be estimated from the sizes of the yolk sacs that the embryos we used were approximately 19–23 months of age. Occasional "spontaneous" birth of pups of captive females was observed in late August. Embryos removed from the uterus were kept in apparently good condition for periods as long as several weeks in large, covered glass dishes (2-liter capacity) in groups of 4 or 5, in slowly flowing sea water. Whenever possible, all pups used in an experiment were taken from the same mother. In experiments requiring large numbers of pups it was necessary to combine litters from several mothers and these were distributed as evenly as possible into the different experimental groups (Table II). The limited number of embryos of equivalent development available at one time, and the limited capacity of the respirometers made it impossible to test all hormones at the same time. For this reason, six different experiments were performed during an eight-week period.

In each experiment groups of embryos were injected intraperitoneally on alternate days with various doses (Table II) of hormones, propylthiouracil, or 0.7% NaCl solution, always in a volume of 0.05 cc. Oxygen consumption was determined on the day after injection to avoid possible responses to handling.

The compounds tested for their effect on oxygen consumption were l-thyroxine (Tx), l-triiodothyronine (3 : 5 : 3′-triiodo-l-thyronine, T_3), triiodothyroacetic acid (3 : 5 : 3′ triiodothyroacetic acid, Triac) in doses of 10 micrograms or 100 micrograms, and propylthiouracil in doses of 50 micrograms.

EXPERIMENTS AND RESULTS

Six experiments (Table III) were completed. The total number of injections, given at 2-day intervals, was as few as 4 or 5, but was usually (in four of the six experiments) 8 to 10. The shorter experiments were ended when accidental blockage of the sea water occurred. Since such occurrences were obviously harmful, and their effects difficult to assay, respiratory measurements were accordingly not continued. In experiment 1 such a blockage killed all Triac-injected animals after the fifth injection (Fig. 2). The Tx-injected animals in this experiment showed an extreme but temporary respiratory depression (Fig. 2) at the same time, so may have had a brief experience of the same nature. Only one other extremely variant datum is seen in Figure 3, which shows the results of experiment 2. Here an exceptionally high respiratory rate was observed in saline solution-injected controls after three injections. Since these same animals in succeeding measurements showed relatively little variation in oxygen consump-

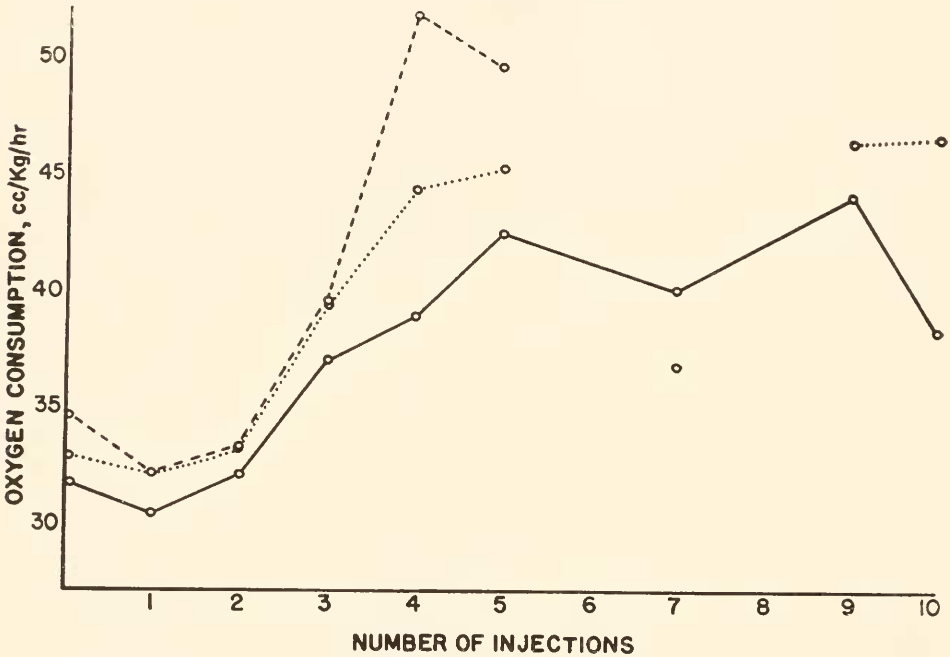

FIGURE 2. The effects of repeated injections of thyroid compounds on metabolic rate of dogfish embryos—injection group 1. Solid line, control; dashed line, triiodothyroacetic acid (10 μg.); dotted line, l-thyroxine (10 μg.).

tion, it is felt that the exceptional figure may have been due to some limited experience, possibly a brief interruption in water supply. The remaining data, summarized in the figures, and Table III, appear to show consistent trends of response, or lack of response, to the various forms of treatment.

It may be seen in Figures 1 and 2, and Table III, that the oxygen consumption of control embryos varied throughout the periods of respiratory measurement, but not in any particular pattern. The nature of this variation, whether due to maturational or environmental factors, is not clear. However, whatever the basis for the variation in control respiratory metabolism, the changes were generally gradual. In the first experiment (Fig. 2) oxygen consumption increased gradually through most of the three-week period of observation; in the second experiment (Fig. 3) this variation was, in general, less and showed no such constant trend.

Fortunately, the general variation of the controls was paralleled by the hormone-injected embryos, and in addition, a relative difference from the controls was usually maintained, if it occurred at all. Accordingly, when the results are expressed as per cent of the control oxygen consumption some conclusions appear to be offered (Table III, Fig. 4).

The most potent stimulator of oxygen consumption in these tests was triiodothyroacetic acid (Fig. 4A). Despite all the variations to which such experiments seem to be prone, in all four experiments in which Triac was injected in 10-microgram quantities, it clearly induced an increase in oxygen consumption to maxima 17% to 33% above the control. These maxima were achieved 8 to 10 days after beginning the injections and thereafter oxygen consumption progressively

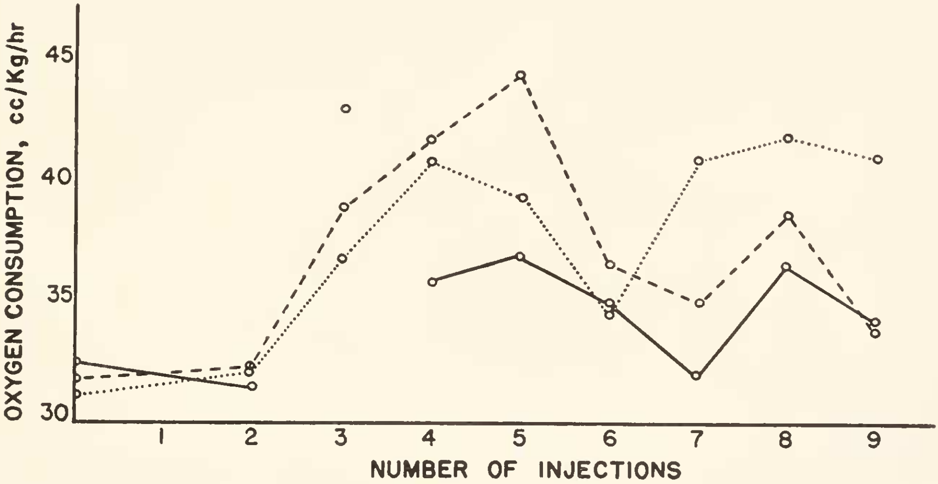

FIGURE 3. The effects of repeated injections of thyroid compounds on metabolic rate of dogfish embryos—injection group 2. Solid line, control; dashed line, triiodothyroacetic acid (10 μg.); dotted line, l-thyroxine (10 μg.).

decreased. The larger dose of Triac (100 micrograms) was less effective than the smaller one (Table III).

Thyroxine, in 10-microgram doses, was not as clearly a stimulator of oxygen consumption in the *Squalus* pups as was Triac. It consistently raised oxygen consumption in two of four experiments (Table III, Fig. 4B) above that of controls, but failed to do so in one, and in another did not produce a significant stimulation until the very end. However, in all thyroxine experiments oxygen consumption was rising at the end of the period of treatment, in comparison with the controls. In one test with 100-microgram quantities of thyroxine a 12% increase above the controls in respiratory metabolism was noted.

Triiodothyronine, in 100-microgram quantities, in all instances stimulated oxygen consumption to levels as high as 18% to 20% above the controls (Table III).

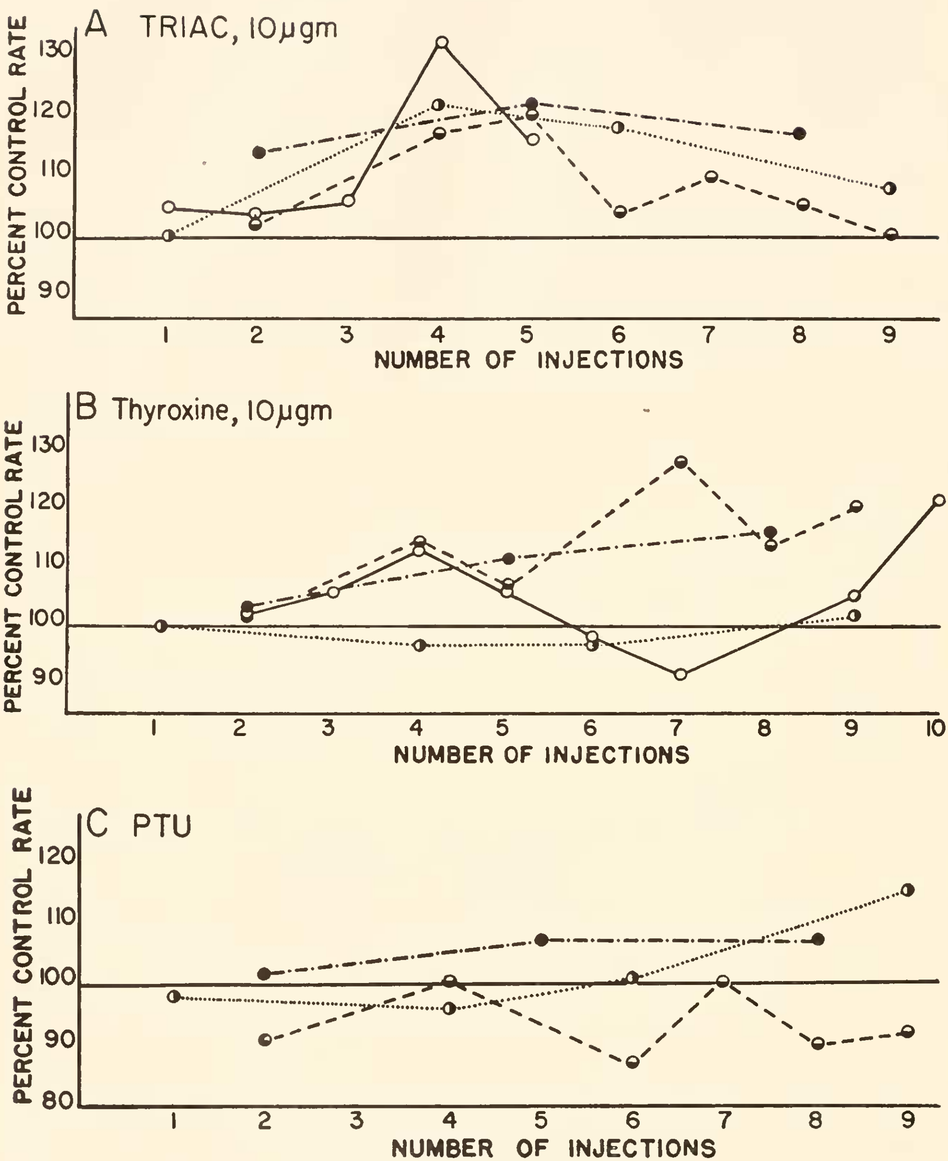

FIGURE 4; A, B, and C. The effects of repeated injections of thyroid compounds on metabolic rate of dogfish embryos. A, triiodothyroacetic acid, 10 μg. each injection; B, l-thyroxine, 10 μg.; C, propylthiouracil, 50 μg. Points represent per cent of the control rate. Open circles, injection group 1; horizontal-barred circles, injection group 2; vertical-barred circles, injection group 3; filled circles, injection group 4.

Propylthiouracil had no particular effect on oxygen consumption relative to the saline-injected controls (Table III, Fig. 4C).

Discussion

Results of this investigation indicate that, under the conditions of these experiments, 10 micrograms of triiodothyroacetic acid (Triac), given on alternate days, have acted as a stimulant of oxygen consumption in the near-term shark embryo (Fig. 4A). The unusual feature of this response is its diminution after 8 to 10 days despite continued injections of the hormone. In laboratory mammals (Foster, Palmer and Leland, 1936) and in clinical use (Means, Lerman and Salter, 1933) the continued administration of thyroxine or crude thyroid preparations is usually accompanied by a sustained increased respiratory rate. However, even in clinical experience it has been reported (Eppinger and Salter, 1935) that an initial rise in metabolism following a week of treatment with thyroid hormone may be followed by a sharp drop, even though treatment is continued. Thus, this pattern of metabolic response is not unprecedented, and may depend on particular physiological factors involved in the response to thyroid hormone. It is of especial interest that Triac has been found to be about 10 to 25 times as active as thyroxine in stimulating amphibian metamorphosis (Pitt-Rivers and Tata, 1959). A ten-fold larger dose of Triac was no more active than the 10-microgram quantity, and appeared, in fact, less active (Table III).

Thyroxine, in either the 10- or 100-microgram dosage, was less clearly a metabolic stimulant. In two of four experiments animals receiving the 10-microgram dose remained consistently higher than controls in oxygen consumption by some 10% to 20%. In the other two experiments this superiority was either lacking or irregularly variable. However, in all four instances oxygen consumption was rising (relative to the saline-injected controls) at the ends of the experiments (Fig. 4B). This was the most variably effective metabolic stimulant and no explanation can be offered for this variability. Larger doses of thyroxine and triiodothyronine (Table III) produce a 12 to 20% increase in oxygen consumption by 10 to 16 days after beginning the injections of hormone.

Propylthiouracil was neither a stimulant nor depressant for oxygen consumption in four different experiments which lasted about 18 days each. Almost all measures of oxygen consumption in dogfish pups treated with this antithyroid drug were within 10% of the control. It has been reported by Zaks and Zamkova (1952) that thiourea consistently reduces the oxygen consumption of young salmon and sturgeons below that of controls. Chavin and Rossmore (1956), working with thiouracil-treated young goldfish, found no effect on oxygen consumption. Interpretation of results of treatment with antithyroid drugs is always complicated by the fact that they are known to be toxic, even in small doses. Since the absence of a metabolic response to propylthiouracil might mean merely that no hormone is yet produced by the thyroid of these embryonic animals, five of them were injected with radioiodide (5 microcuries) and the rest were fixed for histological examination to investigate this possibility. The 24-hour thyroidal radioiodine uptake varied from 0.25% to 2%, a small but significant degree of accumulation. This compares favorably with thyroid uptakes of about 1.5% found by Gorbman and Waterman (unpublished) in pups of the Atlantic spiny dogfish, *Squalus acanthias.* Vivien and Rechenmann (1954) who also treated shark pups (*Scyliorhinus canicula*) with I^{131}, observed by radioautographic techniques that it is deposited in the thyroid, presumably in protein-bound form. The thyroid tissue examined histologically showed a slight increase in average cell height (about 25%) and "vacuolization" of the colloid. This would appear to indicate that the pituitary-thyroid axis of mutual responsiveness is differentiated in these animals, and that it had responded in the PTU-treated animals to a change in thyroid hormone output by TSH secretion. However, despite this apparent decrease in endogenous thyroid hormone production there was no detectable change in oxygen consumption. It is possible that this decrease in endogenous thyroid hormone, if real, was much smaller in size than the 10 micrograms in the injected dose.

In summary, it may be said that triiodothyroacetic acid has been shown in these experiments to be a temporary stimulant of oxygen consumption in near-term embryos of *Squalus suckleyi.* Triiodothyronine proved slightly less active, and the metabolic stimulation by thyroxine was irregular. The thyroid glands of these animals appeared to be functioning at a low rate, and interruption of this function by propylthiouracil had no demonstrable effect on oxygen consumption.

Summary

1. The oxygen consumption rate of "near-term" pups of the dogfish, *Squalus suckleyi,* was determined at regular intervals during the course of repeated injections of physiological saline solution, thyroid hormones, or of anti-thyroid substances. Up to 10 injections were given on alternate days.

2. Of the compounds tested, triiodothyroacetic acid at a dosage level of 10 micrograms per injection was the most consistent in raising the level of oxygen consumption. The effect, however, was transitory with oxygen consumption rising to a maximum (17% to 33%) level above the saline-injected controls after four injections, thereafter declining slowly to control levels.

3. L-thyroxine at a dosage level of 10 micrograms had a variable effect on oxygen consumption. In two of four experiments the oxygen consumption rate rose irregularly, reaching a level about 20% above the controls after 9–10 injections. In the remaining experiments, there was no clear tendency to remain above the controls.

4. Propylthiouracil, after 9 injections, had no consistent effect on metabolic rate in four experiments.

5. The results are discussed with reference to the possible level of thyroid function in these animals.

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